



**NTP**  
National Toxicology Program

# Phthalate Initiative: Research Concept and plans for future work on Di(2-ethyl)hexyl phthalate (DEHP) and Phthalate Mixtures

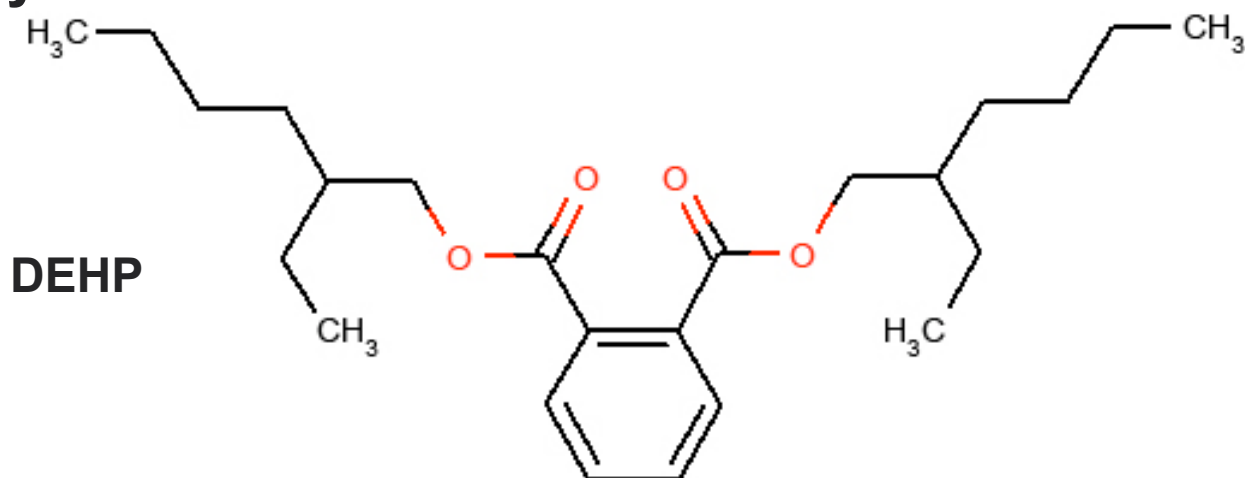
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NTP Board of Scientific Counselors  
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## Nomination History



- DEHP and other phthalates have been nominated on a number of occasion to the NTP.
- Nominations related to this proposal include:
  - Peroxisome proliferators (initiated in the 1990's)
  - Nomination of DEHP by FDA (2004)
  - NTP- CERHR critical data needs from DEHP monograph (2006)



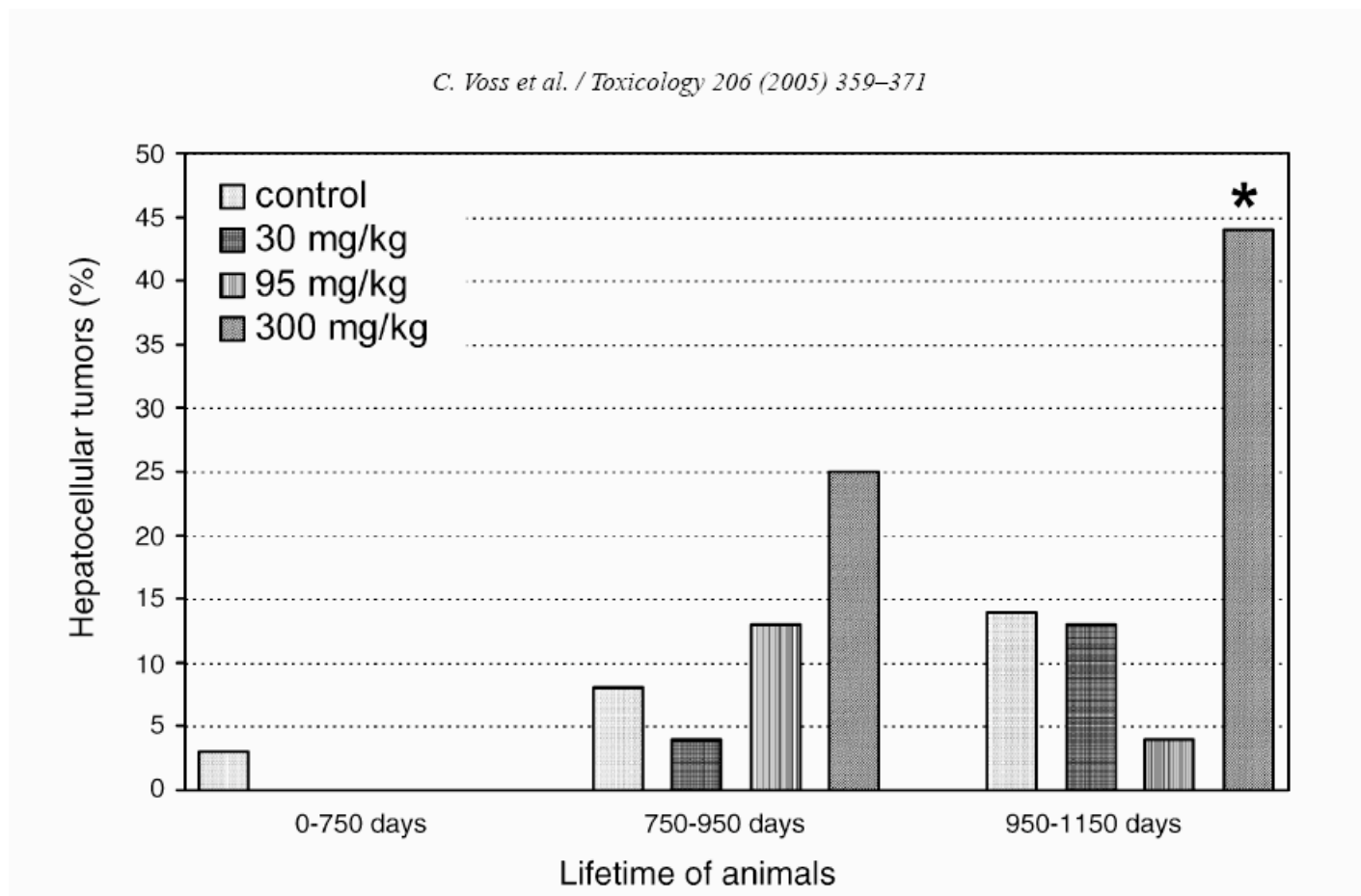
## Background

- DEHP is a ubiquitous environmental contaminant that produces adverse reproductive, developmental and cancer effects in experimental animals.
- Based primarily on NTP bioassays, EPA and IARC designated DEHP as a Category 2 carcinogen in 1992.
- IARC (2000) and the EU (2004) have delisted DEHP as a carcinogen based on mode of action criteria.
  - Liver tumors initiated through a PPAR $\alpha$  (peroxisome proliferator activated receptor – alpha) mechanism that has limited relevance to humans.



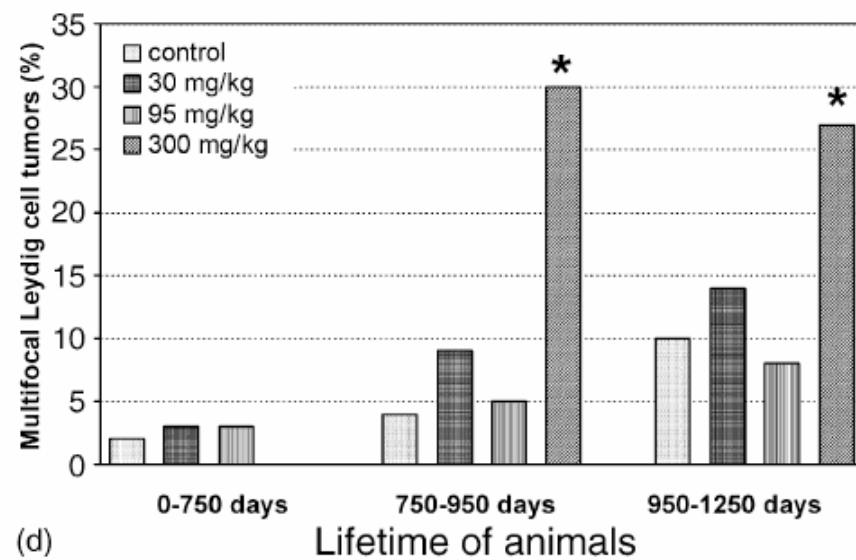
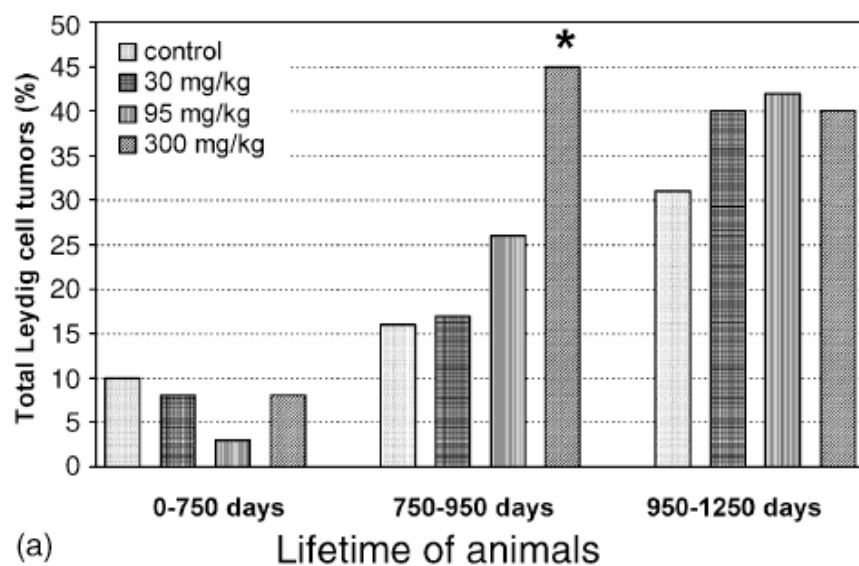
## Background - 2

- More recent lifetime study (Voss *et al* 2005) in the SD rat found liver tumors





## Testicular Leydig cell tumors in Voss study





## Liver Tumors in the PPAR $\alpha$ KO Mouse (22 months DEHP)

	WILD -TYPE			NULL		
Dose (% in diet)	0	0.01	0.05	0	0.01	0.05
#Animals	24	23	20	25	25	31
Hepatocellular Adenoma	0	2	2	0	1	6
Hepatocellular Ca	0	0	0	1	0	1
Choloangio- Ca	0	0	0	0	0	1
<b>TOTAL</b>	<b>0(0%)</b>	<b>2(8.7%)</b>	<b>2(10%)</b>	<b>1(4%)</b>	<b>1(4%)</b>	<b>8(25.8%)*</b>



## Phthalate- induced developmental effects

- Antiandrogenic mode of action.
  - Decreased fetal testicular testosterone levels.
- Male reproductive tract malformations.
- Induction of testis LC tumors and dysgenetic areas after *in utero* only exposure.
- DEHP metabolites in rodent and human (general population) amniotic fluid.
  - MOE ~ 20 (cf 11mg DEHP/kg/d in rat).
- Human exposure data indicates exposure to multiple phthalates that produce developmental effects in rats.
- Recent PFOA study indicates that **post-natal** developmental effects not seen in PPAR $\alpha$  null mouse, but **pre-natal** effects are noted.



## Hypotheses

- That lifetime (perinatal + 2 year) exposure to DEHP would impact the dose response, incidence and/or severity for cancers of the liver and testis (and perhaps pancreas) compared with adult only exposure.
- That PPAR $\alpha$  is developmentally regulated in the rat and **unlikely** to contribute to toxicity initiated *in utero* after exposure to DEHP.
- That exposures to mixtures of phthalates, based on their individual potencies, would result in dose addition for cancer (and other) outcomes.



## Proposed General Approach

- Undertake a DEHP perinatal cancer bioassay in the Wistar (Han) rat.
  - Sensitive window to phthalates
  - More complete assessment of carcinogenic potential
  - Evaluation of targets other than the liver (eg testis, pancreas)
  - Human fetuses are exposed
- Undertake an ontogeny study of PPAR $\alpha$  in the Wistar (Han) rat.
  - When is the receptor first expressed developmentally in phthalate target tissues?
  - Antiandrogenic effects of DEHP (and other phthalates) not found in the mouse i.e. PPAR $\alpha$  null mouse approach would not yield useful information.



## Proposed General Approach

- Undertake perinatal mixture studies using the TEF approach.
- Such studies would require consideration of:
  - Route of exposure and associated kinetics. Estimates of internal dose in the Wistar (Han) rat during pregnancy and lactation by both dietary and gavage routes.
  - Short-term assays on a number of phthalates (e.g. Dibutyl (DBP), Di-isobutyl (DiBP), butylbenzyl (BBP), Di-isononyl (DINP), DEHP [and Diethyl (DEP)] to develop potency estimates in the Wistar (Han) rat.
    - For *in utero* exposures, estimates based on fetal testicular testosterone levels.
    - For weanlings, estimates of hepatic peroxisome proliferator activity (e.g. CYP IVA1, Acyl CoA Oxidase etc).
  - It is anticipated that no more than 3 phthalates would be evaluated in any long-term mixture study.
  - Individual TK data on esters selected to go forward to longer term studies.



## Potential Significance

- Such studies would provide a cancer hazard assessment for lifetime exposure to DEHP.
  - influence of early exposures on cancer outcome.
- Elucidate the developmental ontogeny of PPAR $\alpha$  in the rat and relationship to DEHP-induced cancer (and other developmental toxicity) outcomes.
- Provide toxicity data on important environmental phthalates during lifetime exposures (perinatal + 2 years).
- Provide the critical data to undertake mixture studies using the TEF approach, to inform on potential cumulative and aggregate cancer risk.
- Recent data indicate that because of similar modes of action *in utero*, phthalate esters show dose addition when administered in combination.
  - Appropriate to consider cumulative risk for the class, since human subjects (including fetuses) are typically exposed to multiple phthalates.



## Howdeshell et al Tox Sci 99: 190-202 (2007)

GD 14-18; 500 mg/kg/d

